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SHORT COMMUNICATION

Quinolone Therapy of *Klebsiella pneumoniae* Sepsis following Irradiation: Comparison of Pefloxacin, Ciprofloxacin, and Ofloxacin

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BROOK, I., ELLIOTT, T. B., AND LEDNEY, G. D. Quinolone Therapy of *Klebsiella pneumoniae* Sepsis following Irradiation: Comparison of Pefloxacin, Ciprofloxacin, and Ofloxacin. *Radiat. Res.* 122, 215–217 (1990).

Exposure to whole-body irradiation is associated with fatal gram-negative sepsis. The effect of oral therapy with three quinolones, pefloxacin, ciprofloxacin, and ofloxacin, for orally acquired *Klebsiella pneumoniae* infection was tested in B6D2F1 mice exposed to 8.0 Gy whole-body irradiation from bilaterally positioned ^{60}Co sources. A dose of 10^8 organisms was given orally 2 days after irradiation, and therapy was started 1 day later. Quinolones reduced colonization of the ileum with *K. pneumoniae:* 16 of 28 (57%) untreated mice harbored the organisms, compared to only 12 of 90 (13%) mice treated with quinolones ($P < 0.005$). *K. pneumoniae* was isolated from the livers of 6 of 28 untreated mice, compared to only 1 of 90 treated mice ($P < 0.001$). Only 5 of 20 (25%) untreated mice survived for at least 30 days compared with 17 of 20 (85%) mice treated with ofloxacin, 15 of 20 (75%) mice treated with pefloxacin, and 14 of 20 (70%) treated with ciprofloxacin ($P < 0.05$). These data illustrate the efficacy of quinolones for oral therapy of orally acquired *K. pneumoniae* infection in irradiated hosts. © 1990 Academic Press, Inc.

INTRODUCTION

Ionizing radiation reduces the host's defenses to infection (*1*) and enhances its susceptibility to systemic infection due to endogenous and exogenous organisms (*2, 3*). *Klebsiella pneumoniae* is one of the most frequent causes of gram-negative bacteremia (*4, 5*) and is especially prevalent in immunocompromised patients (*6*).

Therapy for severe systemic infection due to gram-negative bacteria generally involves the use of aminoglycosides in combination with β-lactam antibiotics (*7*). However, several recently developed quinolone compounds have exhibited high *in vitro* bactericidal activity against most gram-negative bacteria, including *K. pneumoniae* (*8*).

In this study, we evaluated the efficacy of oral therapy with several quinolones in a model of experimental septicemia due to orally ingested *K. pneumoniae* in irradiated mice.

MATERIALS AND METHODS

Animals

Female B6D2F1 mice approximately 10 weeks of age were obtained from Jackson Laboratories (Bar Harbor, ME). All animals were kept in quarantine for about 2 weeks before being transferred to a room with a 12-h light–dark cycle. Representative samples were examined to ensure the absence of specific bacteria and common murine diseases. Animals were maintained at an AAALAC accredited facility in microisolator cages on hardwood chip bedding and provided commercial rodent chow and acidified water (pH 2.2) that was changed to tap water 48 h before irradiation. All experimental procedures were done in compliance with National Institute of Health and Armed Forces Radiobiology Research Institute (AFRRI) guidelines regarding animal use and care.

Cobalt-60 Irradiation

Mice were placed in Plexiglas restrainers and exposed to 8.0 Gy whole-body irradiation at 0.4 Gy/min from bilaterally positioned ^{60}Co sources. Dose determinations were made using a 50-ml AFRRI-designed tissue-equivalent ionization chamber calibrated against a National Institute of Standards and Technology ionization chamber. The dose within the exposure field varied by 3%, as determined by thermal luminescence dosimetry conducted within tissue-equivalent mouse phantoms.

Bacteria

The strain used in this study was a clinical isolate of *K. pneumoniae* with a capsule type 5 (AFRRI No. 7). We have used this strain in previous animal studies (*9*). The organisms were harvested in the logarithmic phase of growth in brain heart infusion (BBL) media. A concentration of 10^7 organisms per 1 ml saline was prepared, and a volume of 0.1 ml was fed to each animal by gavage using a blunt syringe. This number of organisms was used since ingestion of lower number of bacteria did not produce a high mortality rate in the animals.

Antimicrobials

Pefloxacin (Rhone-Poulenc-Sante, Anthony Cedex, France), ciprofloxacin (Miles Inc., West Haven, CT), and ofloxacin (Ortho Pharmaceutical Corp., Raritan, NJ) were obtained from their manufacturers. Standard powder formulations with known potencies were used for *in vitro* and *in vivo* studies. Pefloxacin and ciprofloxacin (25 mg/kg) were administered

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every 12 h in 0.1 ml of water by gavage. In the case of ofloxacin 40 mg/kg was given once every day.

Antimicrobial Serum Concentration

Serum concentrations of the antimicrobials were measured in six mice 1 and 11½ h after oral administration of pefloxacin and ciprofloxacin and 1 and 23½ h after oral administration of ofloxacin on the fifth day of therapy. *Bacillus subtilis* ATCC 6633 was used as a test organism, and Mueller-Hinton agar (pH 7.4) was used as a test agar.

In Vitro Susceptibility

Minimal inhibitory concentrations and minimal bactericidal concentrations were determined in Mueller-Hinton broth that was inoculated with an inoculum of 1.5×10^5 organisms per milliliter from an overnight culture. The values for minimal inhibitory concentration/minimal bactericidal concentration for *K. pneumoniae* used in this study were as follows: pefloxacin, 0.06/0.06 μg/ml; ciprofloxacin, 0.03/0.03 μg/ml; and ofloxacin, 0.06/0.12 μg/ml.

Microbial Methods

Animals challenged with bacteria were observed for mortality and signs of disease for 30 days. Five animals were selected at random for each group on Days 4, 6, 8, 10, and 12 following irradiation. Cultures were also obtained from another group of mice whenever the animals showed signs of serious illness and were moribund. Animals were killed by cervical dislocation. Specimens of livers were processed for microbial cultures. No other organs were processed and no blood samples were obtained, since previous studies showed that liver cultures correlated best with the presence of sepsis (*3*). The livers were removed aseptically and homogenized immediately. The ileum was opened, and ileal content samples were obtained using a swab. The liver and stool specimens were swabbed onto blood and MacConkey agars, and the organisms were identified using conventional methods (*10*).

Experimental Design

Forty-eight hours following irradiation, each mouse was fed 10^8 organisms. This time of feeding was chosen after preliminary data indicated that the animals became susceptible to *K. pneumoniae* sepsis when they were fed gram-negative bacteria 48 h after irradiation (Brook, Elliott, Ledney, unpublished data). The increased susceptibility to sepsis as a result of oral feeding 48 h after irradiation with gram-negative bacteria is similar to what we observed with *Pseudomonas aeruginosa* (*11*). Antimicrobial therapy was initiated 24 h later and was administered for 7 days. A total of 240 mice were included in each of the experiments, and each experiment was done three times. However, since the microbiological and mortality data were consistent, only the data from one experiment are reported. Each experiment was composed of the three antibiotic therapy groups and the untreated control group. Each therapy or control group consisted of 60 mice: 20 were observed for mortality, 30 were used for cultures of liver on the designated days, and 10 were used for cultures of livers of morbid animals.

Statistical Methods

Statistical analyses were done using the Cox-Mantel Test (*12*).

RESULTS

Mortality

Mortality in the groups that received each of the quinolones was significantly less ($P < 0.05$) than that of the untreated mice, but the groups were not different from each other (Fig. 1). Only 25% of the untreated mice survived, compared with 85% of those treated with ofloxacin, 75% of those treated with pefloxacin, and 70% of those treated with ciprofloxacin.

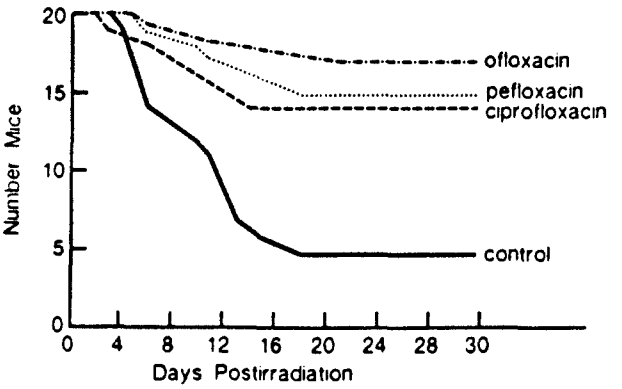

FIG. 1. Survival of γ-irradiated mice B6D2F1 (8.0 Gy) fed 10^8 *K. pneumoniae* and treated orally with three quinolones. The data shown are from one experiment. Two repeated experiments yielded similar results.

Isolation of Organisms in Liver

Klebsiella pneumoniae was isolated in 6 of the 28 (21%) randomly selected untreated animals. There was no correlation between the time following irradiation and the isolation of *K. pneumoniae*. *Klebsiella pneumoniae* was recovered in only 1 of 30 (3%) animals treated with pefloxacin and in none of those treated with ciprofloxacin or ofloxacin ($P < 0.05$). However, *K. pneumoniae* was recovered from 95% of moribund animals of all therapy or control groups.

Isolation of Organisms in Ileal Contents

Klebsiella pneumoniae was isolated in ileal content specimens of 16 of 28 (57%) untreated mice, compared to only 12 of 90 (13%) treated with quinolones ($P < 0.005$). The rate of isolation of *K. pneumoniae* in quinolone-treated mice was 4 of 30 (13%) mice treated with pefloxacin, 5 of 30 (17%) mice treated with ciprofloxacin, and 3 of 30 (10%) mice treated with ofloxacin ($P < 0.005$).

Antibiotic Serum Concentration

Mean serum concentrations of pefloxacin were as follows: 2.4 (±0.3 μg/ml) at 1 h and 0.2 (±0.1 μg/ml) at 11½ h after administration of the antimicrobial. Mean concentrations of ciprofloxacin were 2.8 (±0.5 μg/ml) at 1 h and 0.2 (±0.1 μg/ml) at 11½ h. Mean concentrations of ofloxacin were 2.6 (±0.4 μg/ml) at 1 h and 0.4 (±0.2 μg/ml) at 23½ h.

DISCUSSION

This study demonstrates that the three quinolones, pefloxacin, ciprofloxacin, and ofloxacin, can reduce the col-

onization of the ileum and the development of subsequent septicemia with *K. pneumoniae* in irradiated mice. It supports the findings of Trautmann *et al.* (*13*), who observed the efficacy of ciprofloxacin in the management of systemic *K. pneumoniae* infection in neutropenic mice.

We have developed a model of acquired *K. pneumoniae* infection in irradiated mice that may represent the mode of acquisition of external pathogens into an irradiated host. We have previously shown that irradiated animals develop fatal septicemia due to orally administered *P. aeruginosa* (*11*). We have also observed that the number of the endogenous aerobic and anerobic bacteria in the gastrointestinal tract declined 24 h following irradiation and the decline was maximal at 7 days (*14*). The decrease in the number of that endogenous bacterial flora may make the host more susceptible to the acquisition of external pathogens such as *K. pneumoniae*.

The ability of *K. pneumoniae* to cause systemic infection in irradiated mice may be due to the following factors: the bacterial void created in the gut following the decline in the number of other organisms, the increased permeability of the mucosal cells damaged by irradiation, and the decrease in the local and systemic immune defenses.

The effectiveness of quinolones in the therapy of *K. pneumoniae* infection may be attributed to their local inhibition of the organism's growth within the gut lumen, while preserving the anaerobic gut flora (*15*), and to their systemic antibacterial activity against the organisms that spread to other body sites. The duration of quinolone therapy is yet to be determined. Although minimal mortality occurred following termination of therapy at Day 11, longer therapy might have prevented the mortality noticed after that day. Further studies are underway to determine the optimal duration of therapy.

Selective decontamination of the gut with orally administered quinolones is used to prevent sepsis in immunocompromised hosts (*15, 16*). These agents were also found to be effective in the management of septic episodes in neutropenic patients.[2] The availability of an oral route of administration, the advantage of achieving selective inhibition of potential pathogens in the gut, and the ability to treat systemic infection make the quinolones promising agents for the therapy of irradiated hosts.

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